PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	31 JAN 2005
Applicant's or agent's file reference 701039050025	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US04/00447	07 January 2004 (07.01.2004)	09 January 2003 (09.01.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/68; C12P 19/34; C07H 21/02, 21/04; G01N 33/574; C07K 14/435 and US Cl.: 435/6, 7.1, 91.2; 536/23.1, 23.5, 24.31, 24.33; 530/350, 387.1

Applicant

CHILDREN'S MEDICAL CENTER CORPORATION

1. This opinion contains indications relating to the following items:

- [x] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [x] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [ ] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language ____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☒ in written format

      ☒ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-16	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-16	NO
Industrial applicability (IA)	Claims 1-16	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 1-16 lack an inventive step under PCT Article 33(3) as being obvious over Zetter in view of Yokoyama.
Zetter teaches methods for diagnosing cancer wherein the methods comprise (i) obtaining a sample form a patient; (ii) measuring the level of thymosin beta-15 in the patient sample; and (iii) comparing the level of thymosin beta-15 in the patient sample with a normal control sample, wherein a higher level of thymosin beta-15 expression in the patient sample as compared to the normal, control sample is indicative of cancer (see, e.g., columns 3-4). Zetter teaches that the expression level of thymosin beta-15 can be monitored by analyzing either mRNA or protein levels, using art conventional methods such as RT-PCR or Northern blotting to detect mRNA or ELISA to detect protein (see, columns 5, 6 and 8). The reference further teaches analyzing biological specimens such as blood, tissue, serum, stool, urine, sputum, cerebrospinal fluid and supernatant from cell lysates for the presence of mRNA or protein (see column 5). Additionally, Zetter (column 5) teaches methods for prognosing a patient's cancer by monitoring the level of mRNA or protein. Zetter (column 8) also teaches kits for diagnosing cancer wherein the kits contain antibody or nucleic acid probe reagents for detecting thymosin beta-15. Zetter does not teach diagnosing cancer by detecting thymosin beta-16. However, Yokoyama (see, e.g., Figure 2 and page 318) teaches that human thymosin beta-16 (referred to therein as " NB thymosin beta") is expressed at higher levels in neuroblastoma as compared to control, normal brain cells and teaches that higher levels of thymosin beta-16 are diagnostic of neuroblastoma. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have detected thymosin beta-16 in place of thymosin beta-15 in order to have provided an equally effective means for diagnosing cancers, such as neuroblastomas. With respect to claims 10 and 12, Zetter does not specifically teach detecting mRNA using a microarray or detecting protein by mass spectrometry. However, Zetter (column 5) does teach that any standard technique known in the art for detecting RNA, DNA or proteins can be readily applied to the diagnostic method. In view of the conventionality in the art of microarrays and mass spectrometry, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have used microarrays to detect thymosin beta-16 mRNA or mass spectrometry analysis to detect thymosin beta-16 protein because this would have provided an equally effective means for monitoring thymosin beta-16 expression as a means for diagnosing cancer. With respect to claims 15 and 16, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have packaged the thymosin beta-16 probes and antibodies in a kit for the benefits of convenience and cost-effectiveness for practioners in the art wishing to detect thymosin beta-16 expression and/or wishing to diagnose cancer, such as neuroblastoma.

Claims 1-16 lack an inventive step under PCT Article 33(3) as being obvious over Zetter in view of Shou.
Zetter teaches methods for diagnosing cancer wherein the methods comprise (i) obtaining a sample form a patient; (ii) measuring the level of thymosin beta-15 in the patient sample; and (iii) comparing the level of thymosin beta-15 in the patient sample with a normal control sample, wherein a higher level of thymosin beta-15 expression in the patient sample as compared to the normal, control sample is indicative of cancer (see, e.g., columns 3-4). Zetter teaches that the expression level of thymosin beta-15 can be monitored by analyzing either mRNA or protein levels, using art conventional methods such as RT-PCR or Northern blotting to detect mRNA or ELISA to detect protein (see, columns 5, 6 and 8). The reference further teaches analyzing biological specimens such as blood, tissue, serum, stool, urine, sputum, cerebrospinal fluid and supernatant from cell lysates for the presence of mRNA or protein (see column

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

5). Additionally, Zetter (column 5) teaches methods for prognosing a patient's cancer by monitoring the level of mRNA or protein. Zetter (column 8) also teaches kits for diagnosing cancer wherein the kits contain antibody or nucleic acid probe reagents for detecting thymosin beta-15. Zetter does not teach diagnosing cancer by detecting thymosin beta-16. However, Shou (Table 1 and pages 2833-2844) teaches that human thymosin beta-16 (referred to therein as "N B thymosin beta") is expressed at higher levels in tumorigenic prostate BPH cells and prostate cancer cells verus normal epithelial cells and teaches that higher levels of thymosin beta-16 are diagnostic of prostate cancer and malignancy. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have detected thymosin beta-16 in place of thymosin beta-15 in order to have provided an equally effective means for diagnosing cancers, such as prostate cancer. With respect to claims 10 and 12, Zetter does not specifically teach detecting mRNA using a microarray or detecting protein by mass spectrometry. However, Zetter (column 5) does teach that any standard technique known in the art for detecting RNA, DNA or proteins can be readily applied to the diagnostic method. In view of the conventionality in the art of microarrays and mass spectrometry, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have used microarrays to detect thymosin beta-16 mRNA or mass spectrometry analysis to detect thymosin beta-16 protein because this would have provided an equally effective means for monitoring thymosin beta-16 expression as a means for diagnosing cancer. With respect to claims 15 and 16, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have packaged the thymosin beta-16 probes and antibodies in a kit for the benefits of convenience and cost-effectiveness for practioners in the art wishing to detect thymosin beta-16 expression and/or wishing to diagnose cancer, such as prostate cancer.

Claims 1-16 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.